

*THIS OPINION WAS NOT WRITTEN FOR PUBLICATION*

The opinion in support of the decision being entered today (1) was not written for publication in a law journal and (2) is not binding precedent of the Board.

Paper No. 48

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS  
AND INTERFERENCES

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*Ex parte* TOSHIO KURIHARA, TOSHIYUKI NISHIO, HISAO YAMAMOTO,  
MINORU KAMIMURA, SHINICHI TESHIMA,  
TSUNEO HANYU and SHIGENORI EMI

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Appeal No. 1996-1960  
Application No. 07/975,167

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HEARD: March 21, 2000

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Before WILLIAM F. SMITH, ELLIS, and SPIEGEL, *Administrative Patent Judges*.  
SPIEGEL, *Administrative Patent Judge*.

*DECISION ON APPEAL*

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 13 through 20, which are all of the claims pending in this application. A copy of claims 13-20 is attached to this decision.

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The references relied on by the examiner are:

Farnham et al. (Farnham)	4,147,860	Apr. 3, 1979
McCroskey	4,472,499	Sep. 18, 1984
Rauscher et al. (Rauscher)	4,709,020	Nov. 24, 1987
Blair	4,794,078	Dec. 27, 1988
Kasahara et al. (Kasahara) <sup>1</sup> (published Kokai patent application)	60-233560	Nov. 20, 1985

The references relied on by the appellants are:

Matsui et al. (Matsui), "Method for Determination of Amylase Activity," 7 *Analysis of Clinical Specimen* 2, English language translation of Figure 12 on page 34 only (1984).<sup>2</sup>

Toyo Boseki Kabushiki Kaisha product insert (Toyo), DIACOLOR LIPASE, partial English language translation of excerpts from pages 2-3 (date unknown).<sup>3</sup>

Claims 13-20 stand rejected under 35 U.S.C. § 103 as being unpatentable over Kasahara, McCroskey, Farnham and either Rauscher or Blair. We REVERSE.

In reaching our decision in this appeal, we have given careful consideration to the appellants' specification and claims and to the respective positions articulated by the appellants and the examiner.

We make reference to the examiner's answer (Paper No. 37, mailed

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<sup>1</sup> We refer in our decision to a translation of Kasahara provided to the PTO by Ralph McElroy Translation Company dated April 1996, a copy of which is attached to our decision.

<sup>2</sup> Copy of Japanese language article and partial English translation supplied as an attachment to appellants' reply brief (Paper No. 39, filed June 13, 1995).

<sup>3</sup> Copy of Japanese language product insert and partial English translation supplied as an attachment to appellants' reply brief (Paper No. 39, filed June 13, 1995).

April 13, 1995), to the examiner's supplemental answer (Paper No. 40, mailed August 29, 1995) and to the examiner's second supplemental answer (Paper No. 42, mailed January 23, 1996) for the examiner's reasoning in support of the rejection and to the appellants' brief (Paper No. 36, filed January 4, 1995), to the appellants' reply brief (Paper No. 39, filed June 13, 1995) and to the appellants' second reply brief (Paper No. 41, filed October 30, 1995) for the appellants' arguments thereagainst.<sup>4</sup>

The claimed invention is directed to

- C water-soluble  $G \sim OR_3$  sugar ester derivatives *per se* which are 4-nitrophenyl or 4-(nitrovinyl)phenyl-glucosides (i.e., formula (I)(B)(C)) or 4-nitrophenyl or 4-(nitrovinyl)phenyl-maltosides (i.e., formula (I)(A)(C)) in which the hydroxyl group(s) at the 4- and/or 6-position of the non-reductive terminal glucose is modified with an ester residue of saturated or unsaturated fatty acid having 5-30 carbon atoms (claims 13-14),
- C reagents for measuring lipase activity comprising the  $G \sim OR_3$  sugar ester derivative (claims 15 and 19), further comprising at least one auxiliary enzyme selected from  $\alpha$ -glucosidase, glucoamylase and  $\beta$ -glucosidase (claims 16-18), and
- C a method for measuring lipase activity comprising allowing a sample to act on the  $G \sim OR_3$  sugar ester derivative in the presence of at least one auxiliary enzyme selected from  $\alpha$ -glucosidase, glucoamylase and  $\beta$ -glucosidase to produce cleaved 4-nitrophenol or 4-(nitrovinyl)phenol compounds, which compounds are then measured to determine the lipase activity of the sample (claim 20).

(See specification, pages 4-5 and 13-14; brief, pages 2-4.)

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<sup>4</sup> Appellants' third reply brief (Paper No. 43, filed March 25, 1996) was refused entry by the examiner in a letter mailed May 8, 1996 (Paper No. 44) "because it is not limited to new points of argument or to new grounds of rejection raised in the examiner's answer" and, therefore, has not been considered in our decision.

### *OPINION*

Kasahara describes methods and reagents for measuring lipase activity characterized by using a water-soluble glyco-fatty acid ester substrate, wherein the fatty acid ester residue has about 5-22 carbon atoms and is bonded on the hydroxy groups of a mono-, oligo- or poly-saccharide, e.g., glucose, maltose or starch (page 3, lines 17-30; page 4, lines 1-2 and 9-11), e.g., at the hydroxyl group(s) at the 1- and/or 6-position of the saccharide terminal (page 4, lines 9-15). Examples include 6-O-palmitoyl glucose (page 4, lines 17-18) and 6-O-oleyl maltose (page 6, line 23). Lipase cleaves the substrate into its constituent saccharide and fatty acid (page 4, lines 20-22) and either the amount of the saccharide or fatty acid formed is determined as a measurement of lipase activity (page 4, lines 29-30). For example, if the saccharide is maltose, the amount of maltose formed by the lipase reaction may be quantitated by reacting the maltose with maltase (i.e.,  $\alpha$ -glucosidase) to form glucose, which glucose is then oxidized by glucose oxidase to produce hydrogen peroxide which is then measured colorimetrically by a 4-aminoantipyrine-phenol-peroxidase reaction (page 5, lines 1-6; page 9, penultimate line and pages 8-9, *Application Example 2*).

McCroskey describes temperature-independent methods and reagents for determining the activity of enzymes which can hydrolyze substrates capable of releasing p-nitrophenol (col. 1, lines 31-

49; col. 3, lines 36-38). Such substrates include p-nitrophenyl derivatives of mono and oligosaccharides, wherein in the case of oligosaccharides, the p-nitrophenol radical is attached to the terminal glucose unit (col. 2, lines 16-19). Preferred substrates include (a) p-nitrophenyl-"-maltopentaoside and p-nitrophenyl-"-maltohexaoside for determining "-amylase activity, (b) p-nitrophenyl-"-glucoside and p-nitrophenyl-"-maltoside for determining "-glucosidase activity and (c) p-nitrophenyl- $\beta$ -glucoside and p-nitrophenyl- $\beta$ -maltoside for determining  $\beta$ -glucosidase activity (col. 2, lines 38-47).

Farnham describes methods for preparing nitroaromatic glycosides useful as substrates in determining "-amylase activity (col. 1, lines 14-18), comprising a series of acetylation, nitration and deacetylation steps (col. 1, line 65 - col. 2, line 54). The nitroaromatic residue may be substituted with halogen (col. 2, lines 14-34).

Blair describes "-amylase assays using oligosaccharide substrates of 4-10 glucose units with a chromogen, e.g., p-nitrophenol, on the reducing end, wherein the substrate is cleaved into smaller fragments by "-amylase (an endo-enzyme) and the smaller fragments are cleaved with an exo-enzyme, e.g., "-glucosidase,  $\beta$ -glucosidase or glucoamylase, to liberate p-nitrophenol (col. 1, lines 9-24). Blair discloses an "-amylase substrate comprising an oligosaccharide having at least 3 glucose units, its reducing end glucose bonded, via a bond cleavable by "- or  $\beta$ -glucosidase, to a chromogen and its non-reducing end or terminal glucose unit bonded to a blocking group which inhibits cleavage by exo-

enzymes (col. 1, lines 49-64) so that in the absence of  $\alpha$ -amylase no color change will occur in the substrate (Fig. 3; col. 2, lines 17-20). Suitable blocking groups include carboxylic acid esters, e.g., acetyl or benzoyl radicals (col. 4, lines 9-12).

Rauscher also describes bonding blocking groups (i.e., R and R<sub>1</sub>, each independently a straight-chained or branched alkyl or alkoyl radical containing up to 6 C atoms or a phenyl radical or together forming a methylene bridge, the H atoms of which can be substituted by an alkyl radical containing up to 5 C atoms or a phenyl radical) on the 4- and 6-hydroxy groups of the terminal glucose unit of a nitrophenylligoglucoiside (col. 1, lines 41-61) to provide an  $\alpha$ -amylase substrate which is storage-stable in the presence of  $\alpha$ -glucosidase (col. 1, lines 37-39).

According to the examiner,

[i]t would have been obvious to a person of ordinary skill in the art at the time the invention was made to block the terminal glucose units of the compounds of McCroskey with a fatty acid ester because such a group would be expected to prevent glucosidase activity (Blair or Rauscher et al.) until such time as its removal by lipase (Kasahara) and under the conditions normally employed in such assays a measure of lipase activity would be obtained. [Answer, page 6, last para.]

However, McCroskey discloses p-nitrophenyl- $\alpha$ /β-glucoside and p-nitrophenyl- $\alpha$ /β-maltoside as substrates **for** determining  $\alpha$ /β-glucosidase activity. Blocking the terminal glucose units of these substrates with any blocking group “to prevent glucosidase activity” would destroy the very purpose for

which these substrates were made.<sup>5</sup> Moreover, the examiner has failed to point out and we do not find where McCroskey, Farnham, Blair and/or Rauscher disclose or suggest either substrates for measuring lipase activity or C<sub>5</sub>-C<sub>30</sub> fatty acids as blocking groups. Therefore, while the references *could* be combined as the examiner argues, the examiner has failed to provide a coherent reason why the references *should* be combined. The mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification. *In re Laskowski*, 871 F.2d 115, 117, 10 USPQ2d 1397, 1398-99 (Fed. Cir. 1989); *In re Gordon*, 733 F.2d 900, 902, 221 USPQ 1125, 1127 (Fed. Cir. 1984).

We note that the examiner appears to have changed his position in his response to appellants' arguments, i.e., that "the person of ordinary skill in the art at the time the invention was made would have recognized the advantages of the p-nitrophenol substrates and would have substituted them for the polysaccharides of Kasahara" (answer, page 8, para. 3 and page 13, top para.); that "[t]he real world clinical chemist recognizes that nitrophenol substituted saccharides are functional equivalents of saccharides for the determination of serum enzymes" (answer, page 11, para. 2); and, "there is ample ***motivation*** in the prior art to replace unsubstituted saccharides with saccharides containing p-nitrophenol is [*sic*, as] set forth in Farnham et al. in the section entitled 'Background of the Invention'

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<sup>5</sup> The claimed invention has been limited to recite G ~ OR<sub>3</sub> sugar esters having only one or two glucose units, i.e., glucoside or maltoside derivatives. According to the examiner, "[i]t is important to note that the invention originally claimed included substrates with sufficient glucose units so that they would function as amylase" (answer, page 6, fn 2). The examiner's position began to fall apart when the claims were so narrowed.

wherein it is clearly set forth that such substrates avoid the problem of interference from the presence of saccharides normally present in the clinical sample” (answer, page 12, para.4). Appellants argue that although Kasahara has compounds which meet the formula “G” of claim 13, Kasahara does not disclose the required p-nitrophenol group and it is *impossible* to modify Kasahara to add a *hydrophobic* p-nitrophenol group because Kasahara requires a *water-soluble* substrate (brief, pages 8-10).

In rejecting claims under 35 U.S.C. § 103, it is incumbent upon the examiner to establish a factual basis to support the legal conclusion of obviousness. *In re Fine*, 837 F.2d 1071, 1073, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988). The examiner has not pointed out, and we do not find, where Kasahara, McCroskey, Farnham, Rauscher and/or Blair discloses or suggests that p-nitrophenol-glucosides/maltosides esterified at the 4- and/or 6-position of the non-reductive terminal glucose with a fatty acid of 5 to 22 (Kasahara) or 30 (claimed invention) carbon atoms would have reasonably been considered to be either water-soluble compounds or functional equivalents of Kasahara’s glyco-fatty acid ester substrates. Although the examiner argues that any one of McCroskey, Farnham, Rauscher or Blair shows that the substrates are water soluble (answer, para. bridging pages 11-12), none of these four references disclose p-nitrophenyl substituted substrates *esterified with a fatty acid of 5 to 22 or 30 carbon atoms*. The examiner also relies on “the textbook teachings of Morrison et al. ... [wherefrom a] simple calculation indicates that approximately 0.12 moles of p-nitrophenol is [sic]

soluble in 1 liter of water” (answer, page 12, first full para.). Ordinarily, when a reference is relied on to support a rejection even in a "minor capacity," that reference should be positively included in the statement of rejection. *In re Hoch*, 428 F.2d 1341, 1342 n.3, 166 USPQ 406, 407 n.3 (CCPA 1970). The examiner has not cited Morrison as part of the rejection. However, even if he had, Morrison is even less persuasive than McCroskey, Farnham, Rauscher or Blair because Morrison only discloses p-nitrophenol devoid of *any* saccharide component.

For the above reasons, we find the examiner has not carried his burden of establishing a *prima facie* case of obviousness. Having concluded that the examiner has not established a *prima facie* case of obviousness, we do not reach the rebuttal references and declaratory evidence discussed in appellants' brief (page 9, fn 1) and reply brief (pages iii-iv).

The rejection of claims 13-20 under 35 U.S.C. § 103 as being unpatentable over Kasahara, McCroskey, Farnham and either Rauscher or Blair is reversed.

## CONCLUSION

To summarize, the decision of the examiner to reject claims 13-20 under 35 U.S.C. § 103 as being unpatentable over Kasahara, McCroskey, Farnham and either Rauscher or Blair is reversed.

**REVERSED**

WILLIAM F. SMITH )  
Administrative Patent Judge )

Appeal No. 1996-1960  
Application No. 07/975,167

JOAN ELLIS  
Administrative Patent Judge

CAROL A. SPIEGEL  
Administrative Patent Judge

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Application No. 07/975,167

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# APPENDIX

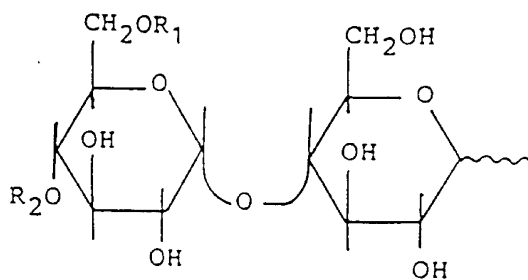
13. A sugar ester of fatty acid represented by the formula

(I)

$G \text{---} O \text{---} R_3$

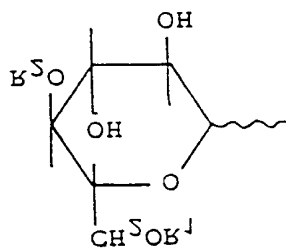
(I)

wherein G stands  
 formula (A)



for a group of the  
 (A)

or a group of the



(B)  
 formula (B)

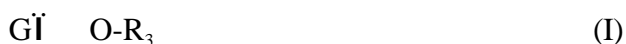
wherein at least one of  $R_1$  and  $R_2$  means an ester residue of a saturated or unsaturated fatty acid having 5 - 30 carbon atoms and the remainder means hydrogen atom or acetyl group and  $R_3$  means a group of the formula (C)

wherein X means a halogen atom, m means an integer of 0 to 4, Y means hydroxy group, an alkoxy group, a carboxyl group or sulfonic acid group, n means 0 or 1 and Z means nitro group or nitrovinyl group.

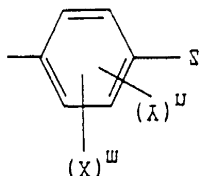
14. A compound as claimed in claim 13 which is selected from among the following compounds:

- (1) 2-Fluoro-4-nitrophenyl 6-O-oleoyl- $\beta$ -D-glucopyranoside
- (2) 4-Nitrophenyl 6-O-palmitoyl- $\beta$ -D-glucopyranoside
- (3) 4-Nitrophenyl 4-O-acetyl-6-O-linoleoyl-0- $\beta$ -D-glucopyranosyl-(1 $\beta$ 4)- $\beta$ -D-glucopyranoside
- (4) 2,3-difluoro-4-nitrophenyl 6-O-lauroyl-0- $\beta$ -D-glucopyranosyl-(1 $\beta$ 4)- $\beta$ -D-glucopyranoside
- (5) 4-Nitrophenyl 6-O-pentanoyl-0- $\beta$ -D-glucopyranoside
- (6) 4-Nitrophenyl 4, 6-D-dipentanoyl-0- $\beta$ -D-glucopyranoside.

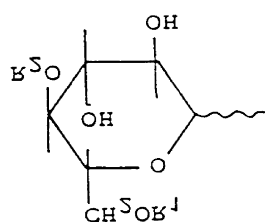
15. A reagent for measuring lipase activity which is characterized by containing, as the substrate, a sugar ester of fatty acid represented by the formula (I)



wherein G

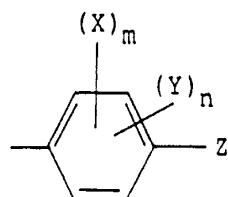


stands for a group of the formula (A)  
(C)



(B)

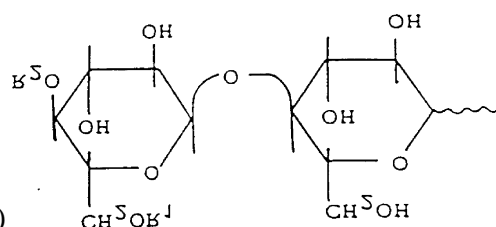
or a group



of the formula (B)

(C)

wherein at  
 an ester  
 unsaturated  
 atoms and the  
 atom or acetyl  
 the formula (C)



(V)

least one of  $R_1$  and  $R_2$  means  
 residue of a saturated or  
 fatty acid having 5 - 30 carbon  
 remainder means hydrogen  
 group and  $R_3$  means a group of

wherein X means a halogen atom, m means an integer of 0 to 4, Y means hydroxy group, an alkoxy group, a carboxyl group or sulfonic acid group, n means 0 or 1 and Z means nitro group or nitrovinyl group.

16. A reagent for measuring lipase activity comprising a substrate as claimed in claim 15 and an auxiliary enzyme.

17. A reagent for measuring lipase activity as claimed in claim 16 wherein the sugar ester of fatty acid is a compound in which the aglycon is bonded in the % -type, and the auxiliary enzyme is % -glucosidase alone or a combination of % -glucosidase and glucoamylase.

18. A reagent for measuring lipase activity as claimed in claim 16 wherein the sugar ester of fatty acid is a compound in which the aglycon is bonded in the \$ -type and the auxiliary enzyme is \$ -glucosidase alone, a combination of % -glucosidase and \$ -glucosidase or a combination of % -glucosidase, glucoamylase and \$ -glucosidase.

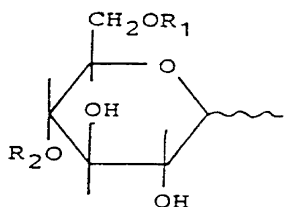
19. A reagent for measuring lipase activity as claimed in claim 15 in which the sugar ester of fatty acid represented by the formula (I) is selected from among the following compounds:

- (1) 2-Fluoro-4-nitrophenyl 6-0-oleoyl-\$-D-glucopyranoside
- (2) 4-Nitrophenyl 6-0-palmitoyl-% -D-glucopyranoside
- (3) 4-Nitrophenyl 4-0-acetyl-6-0linoloyl-0-% -D-gluco-pyranosyl-(164)-% -D-glucopyranoside
- (4) 2,3-difluoro-4-nitrophenyl 6-0-lauroyl-0-% -D-gluco-pyranosyl-(164)-\$-D-glucopyranoside
- (5) 4-Nitrophenyl 6-0-pentanoyl-0-% -D-glucopyranoside
- (6) 4-Nitrophenyl 4,6-D-dipentanoyl-0-% -D-glucopyranoside

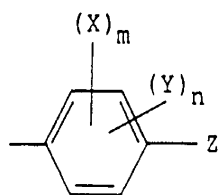
20. A method for measuring lipase activity which comprises allowing a sample to act on a sugar ester of fatty acid represented by the formula (I)



wherein G stands for a group of the formula (A)



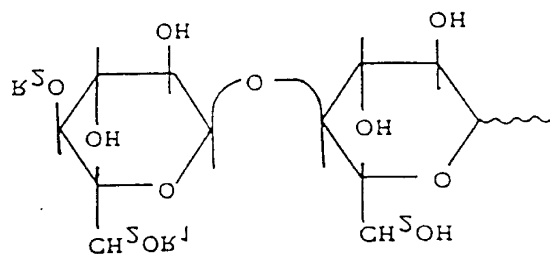
(B)



(C)

or a group of the

formula (B)



(A)

wherein at  
 means an ester  
 unsaturated  
 carbon atoms and the remainder means hydrogen atom or acetyl group, and  $R_3$  means a group of the  
 formula (C)

least one of  $R_1$  and  $R_2$   
 residue of a saturated or  
 fatty acid having 5 - 30